The effects of plant growth regulators on the growth and yields of hydrocarbons in *Helianthus annuus* cv. Munchkin (Asteraceae, Sunflowers)

Robert P. Adams and Sam T. Johnson
Baylor-Utah Lab, Baylor University, 201 N 5500 W, Hurricane, UT, 84737, USA
robert_Adams@baylor.edu

ABSTRACT

Sunflowers, *H. annuus* cv. Munchkin, were subjected to 11 different treatments to determine their effects on the free, stored hydrocarbons (HC) concentrations, leaf biomass, and yields of HC/ g biomass. Biomass was significantly larger than the control for plants subjected to mechanical leaf injury or spraying with benzothiadiazole (1000 ppm). In contrast, spraying with methyl jasmonate (100 µM), gibberellic acid (100 µM), or indole-3-acetic acid (100 µM), resulted in significantly less biomass. Mechanical leaf injury or spraying with Chlormequat Cl (1000 ppm) gave % HC yields the same level as the control. But, % HC yields were significantly lower than the control for plants sprayed with methyl jasmonate (100 µM), gibberellic acid (100 µM), indole-3-acetic acid (100 µM), or Ethephon (100 ppm) (produces ethylene). Total HC yields (as g HC/ g dry wt. 10 lvs.) was correlated with biomass and % HC yields for which mechanical leaf injury or spraying with Chlormequat Cl (1000 ppm) or 2,4-D (100 ppm) resulted in g HC statistically equal to the control plants. However, spraying with methyl jasmonate (100 µM), gibberellic acid (100 µM), indole-3-acetic acid (100 µM), or Ethephon (100 ppm) gave g HC yields significantly lower that the control. Over all, none of the treatments enhanced HC production significantly larger than the control. This may be due to the mixture of chemical classes such as terpenoids, lipids, waxes and sterols that are controlled by genes in various conflicting pathways.
Published on-line www.phytologia.org *Phytologia 101(1): 19-24 (March 21, 2019)*. ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, methyl jasmonate, effects on hydrocarbon yields.

In a seminal paper on the induction of sesquiterpene lactone (STL) defenses in *Helianthus annuus*, by surface application of methyl jasmonate (MeJA), Rowe, Ro and Rieseberg (2012) found that MeJA treated sunflower plants had a lower STL production and lower glandular trichome density. This is in contrast to other studies that have found MeJA to induce increased concentrations of terpenoids in cotton (*Gossypium hirsutum*, Opitz, Kunert and Gershenzon, 2008), *Tanacetum parthenium* (Majdi et al. 2015) and see review on the roles of MeJA in plants by Browse (2005).

It appears that defense chemicals are both constitutive and inducible defenses (see Wittstock and Gershenzon, 2002 for discussion). Recently, we reported (Adams et al. 2017c) that progeny of high hydrocarbon (HC) yielding sunflower (*H. annuus*) populations displayed much reduced HC yields when grown in greenhouse conditions. Notice (Fig. 1) that the percent HC (greenhouse / field grown HC yields) decreased to 45.9, 55.6 and 78.3%. In addition, g HC / g DW leaves was very reduced to from 6.1 to 17.9% in greenhouse grown plants. It appears that biotic and abiotic factors in natural populations can have large effects on HC yields. With this in mind, it seemed of interest to investigate various plant growth regulators on HC yields from greenhouse grown Munchkin, a dwarf sunflower cultivar.

This is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams et al., 1986; Adams and Seiler, 1984; Adams and TeBeest, 2016; Adams et al. 2016; Adams and TeBeest, 2017; Adams et al. 2017a,b,c; Adams et al. 2018a,b,c; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991,).

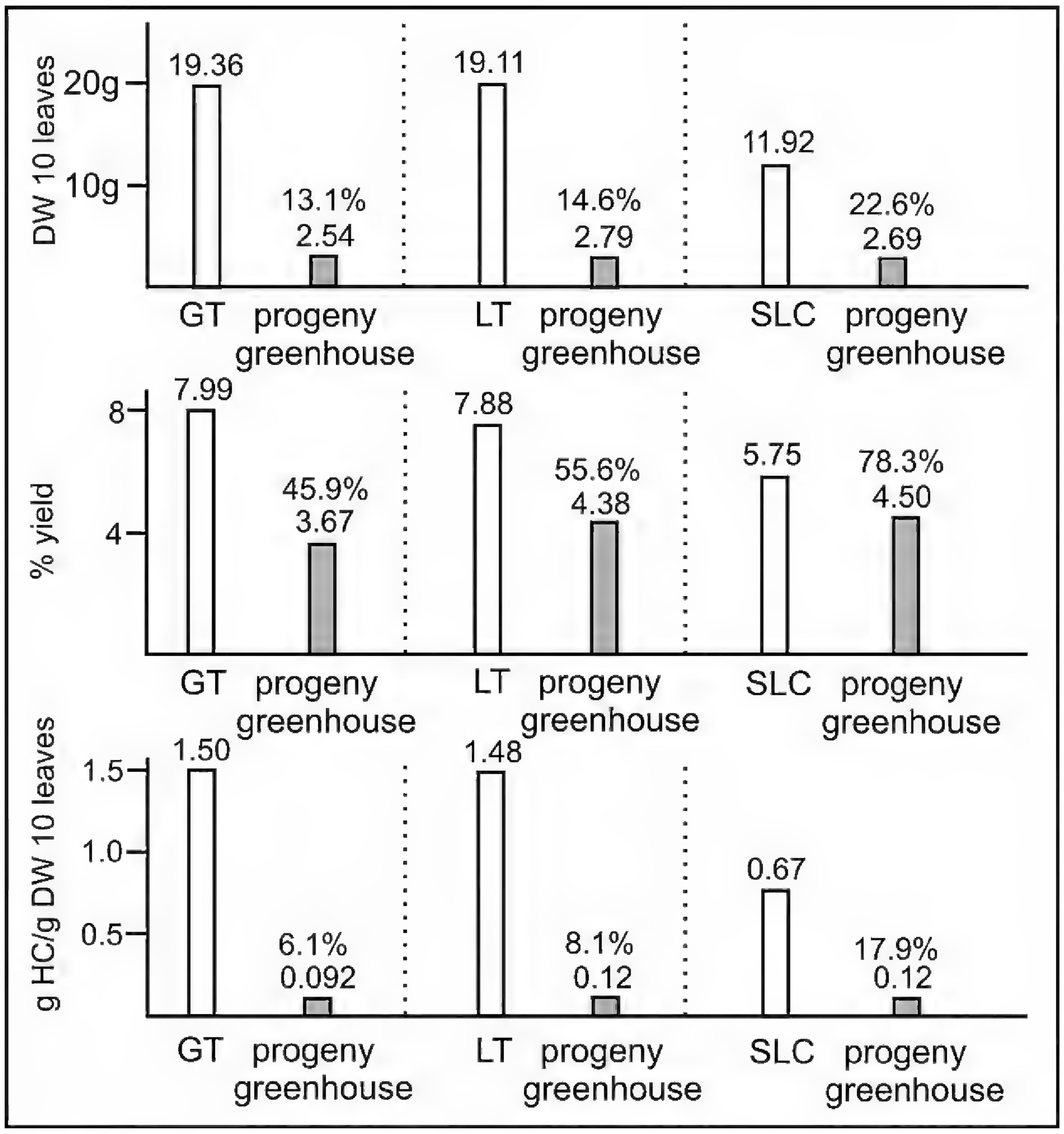

Figure 1. Comparison of DW 10 leaves, HC yield and g HC/ gDW 10 leaves for field sampled sunflowers from Gruver, TX (GT) vs. their progeny grown in the greenhouse at OPSU under ambient natural light (adapted from Adams et al. 2017c).

MATERIALS AND METHODS

Seeds of *H. annuus* cv. Munchkin were obtained from Sunflower Selections, Inc., Woodland, CA. Seeds were planted in 6 " square plastic pots using Miracle Grow® potting soil. Plants were grown in a growth chamber with LED lighting approximately equal to daylight for 16 hr light, 8 hr dark cycles and watered as needed.

The 10 lowest growing, non-yellowed, mature leaves were collected at stage R1 when the first flower but appeared. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air-dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar was sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was filtered through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared. Extraction of identical samples by shaking and soxhlet (8 hr) yielded a correction factor of 1.9 (soxhlet yield/ shaking yield), which when corrected to oven dry weight basis (ODW) by 1.085 resulted in a total correction factor of 2.06.

ANOVA and SNK (Student Newman-Keuls) multiple range tests were programmed following the formulations in Steel and Torrie (1960).

TREATMENTS WITH GROWTH REGULATORS

At the first bud stage, Munchkin plants were treated as shown in Table 1.

Table 1. Treatment of sunflower (cv. Munchkin) with growth regulators and mechanical injury.

Treatment	ref.	Code	Reported effects on plants	# plants
Control		CT		7
Methyl jasmonate (100uM), sprayed	1,2,3	MJ	Increased parthenolide in *Tanacetum parthenium;* xanthumin in *Xanthium*.	7
Mechanical injury w/ cloth wheel, 4 lines	4	IN	Induction of terpenoids in plants.	7
γ-aminobutyric acid GABA,100 ppm	5	AB	Increased defensive enzymes in *Helianthus*.	7
Paclobutrazol 150 ppm	6,7	PB	Growth retardant. Increased pyrethrins in *Chrysanthemum*.	7
BZTD 1000 ppm benzothiadiazole	8	BZ	Induced synthesis of scopoletin in sunflower.	7
2,4-D, 2,4-dichlorophenoxyacetic acid, 100 ppm	9	24D	Accumulation of scopolin in sunflowers. Common tissue culture hormone.	7
Ethephon, (= Floral), 100ppm	6	ET	Decrease apical growth, promote branching. Flower abortion. Delayed flowering	7
Indole-3-acetic acid (IAA), 100 µM	3,8	IA	Increased phenolics in Brassica. Increased xanthumin in *Xanthium*.	7
Gibberellic acid, GA3, 100 µM	3	GA	Increased xanthumin in *Xanthium*.	7
Salicylic acid, 1000 ppm	1,5,8	SA	Increased oil content in *Brassica*. Increased defensive enzymes in *Helianthus*. Increased parthenolide in *Tanacetum parthenium*.	7
Chlormequat chloride, 1000ppm	6,7	CC	Suppresses stem elongation. Increased pyrethrins in *Chrysanthemum*.	7

References cited:

1. Majdi, M., M. R. Abdollahi and A. Maroufi. 2015. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in Tanacetum parthenium. Plant Cell Rep. DOI 10.1007/s00299-015-1837-2.
2. Rowe, H. C., D-K. Ro and L. Rieseberg, 2012 Response of Sunflower (Helianthus annuus L.) leaf surface defenses to exogenous methyl jasmonate. PLoS ONE 7(5): e37191. doi:10.1371/journal.pone.0037191.
3. Li, C-F., F-F. Chen and Y-S. Zhang. 2014. GA_3 and other signal regulators (MeJa and IAA) improve Xanthumin biosynthesis in different manners in *Xanthium strumarium* L. Molecules 19: 12898-12908.
4. Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoid accumulation in Cotton (*Gossypium hirsutum*) foliage is a general wound response. J. Chem. Ecol. 34: 508-522.
5. Usha, D., S. L. Prasad and L. V. Rao. 2016. Effect of biotic and abiotic inducers on induction of defense enzymes in sunflower. Intl. J. of Current Res. 8: 28181-28185.
6. Currey, C. J. and R. G. Lopez. undated. Applying Plant Growth retardants for height control. Commercial Greenhouse and Nursery Production. Purdue University, Extension, doc. HD-248-W.
7. Haque, S., A. H. A. Farooqi, M. M. Gupta, R. S. Sangwan and A. Khan. 2007. Effect of ethrel, chlormequat chloride and paclobutrazol on growth and pyrethrins in Chrysanthemum cinerariaefolium Vis. Plant Growth Regul. 51: 263-269.
8. Thakur, M. and B. S. Sohal. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A Review. ISRN Biochemistry doi.org/10.1155/2013/762412.
9. Dieterman, L. J., C-Y. Lin, L. M. Rohrbaugh and S. H. Wender. 1964. Accumulation of ayapin and scopolin in sunflower plants treated with 2,4-dichlorophenoxyacetic acid. Archives of Biochem. and Biophysics 106: 275-279.

Treatment with growth regulators has had a long history of producing morphological and chemical changes in plants as is shown in the papers referenced in Table 1. Because this study was a screening project, the quantities utilized were those frequently utilized from the literature and not optimized.

RESULTS

Table 2 shows the results from the treatments ANOVA and SNK statistical analyses. Differences for biomass (g dw 10 lvs/ plant) among treatments were very highly significant ($P = 0.37^{-3}$ **). Biomass was significantly larger than the control for mechanical leaf injury or spraying with benzothiadiazole (1000 ppm). In contrast, spraying with methyl jasmonate (100 µM), gibberellic acid (100 µM), or indole-3-acetic acid (100 µM), resulted in significantly less biomass. Mechanical leaf injury or spraying with Chlormequat Cl (1000 ppm) gave % HC yields the same level as the control. But, % HC yields were significantly lower than the control for plants sprayed with methyl jasmonate (100 µM), gibberellic acid (100 µM), indole-3-acetic acid (100 µM), or Ethephon (100 ppm) (produces ethylene). Total HC yields (as g HC/ g dry wt. 10 lvs.) was correlated with biomass and % HC yields for which mechanical leaf injury or spraying with Chlormequat Cl (1000 ppm) or 2,4-D (100 ppm) resulted in g HC statistically equal to the control plants. However, spraying with methyl jasmonate (100 µM), gibberellic acid (100 µM), indole-3-acetic acid (100 µM), or Ethephon (100 ppm) gave g HC yields significantly lower that the control. Over all, none of the treatments enhanced HC production significantly larger than the control. This may be due to the mixture of chemical classes such as terpenoids, lipids, waxes and sterols that are controlled by genes in various conflicting pathways.

Table 2. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for cv. Munchkin, subjected to 12 treatments and analyzed after 4 days. Mean valuess with the same suffix letter are not significantly different (P= 0.05). Treatment codes: see Table 1.

biomass	IN	BZ	24D	AB	CT	ET	PB	SA	CC	GA	IA	MJ	F ratio significance
wt. 10 leaves	3.37 a	3.33 a	3.25 ab	3.21 ab	3.18 ab	3.18 ab	3.14 ab	3.06 ab	3.02 ab	2.90 abc	2.70 bc	2.53 c	F= 3.86 $P = 0.37^{-3}$ ***
treatment/ HC yield	CT	CC	IN	24D	BZ	SA	PB	AB	MJ	IA	GA	ET	F ratio significance
% HC yield	4.66 s	4.48 s	4.45 s	4.28 st	4.12 st	3.96 st	3.88 st	3.70 st	3.26 tu	3.21 tu	3.10 tu	2.49 u	F= 5.76 $P = 0.15^{-4***}$
treatment gHC yield	CT	IN	24D	CC	BZ	SA	AB	PB	GA	IA	MJ	ET	F ratio significance
g HC/ g 10 leaves	.149 x	.149 x	.139 x	.136 x	.134 x	.121 xy	.120 xy	.119 xy	.090 yz	.087 yz	.081 z	.080 z	F= 8.08 $P = 0.117^{-5***}$

Graphing the yields by treatment reveals some interesting patterns (Fig. 2). The control (CT) is highest in % HC yield, gHC/ g10 lvs (biomass), and, statistically, in the highest group for biomass. Mechanical injury (IN) was near the maximum for all variables (Fig. 3). Chlormequat chloride (CC) was near the maximum in both %HC yields and gHC/ g 10 lvs. (Fig. 2).

Several treatments (Ethephon, ET; Gibberellic acid, GA; Indole-3-acetic acid, IA; Methyl jasmonate, MJ) produced significantly lower amounts of %HC yields and gHC/ g10 lvs. (Fig. 2).

Ethephon (ET) and γ-aminobutyric acid (AB) both reduced the %HC yields and gHC/ g 10 lvs, but had little effect on biomass (Fig. 2). Indole-3-aceetic acid (IA) and methyl jasmonate (MJ) both reduce the biomass in only 4 days after treatment.

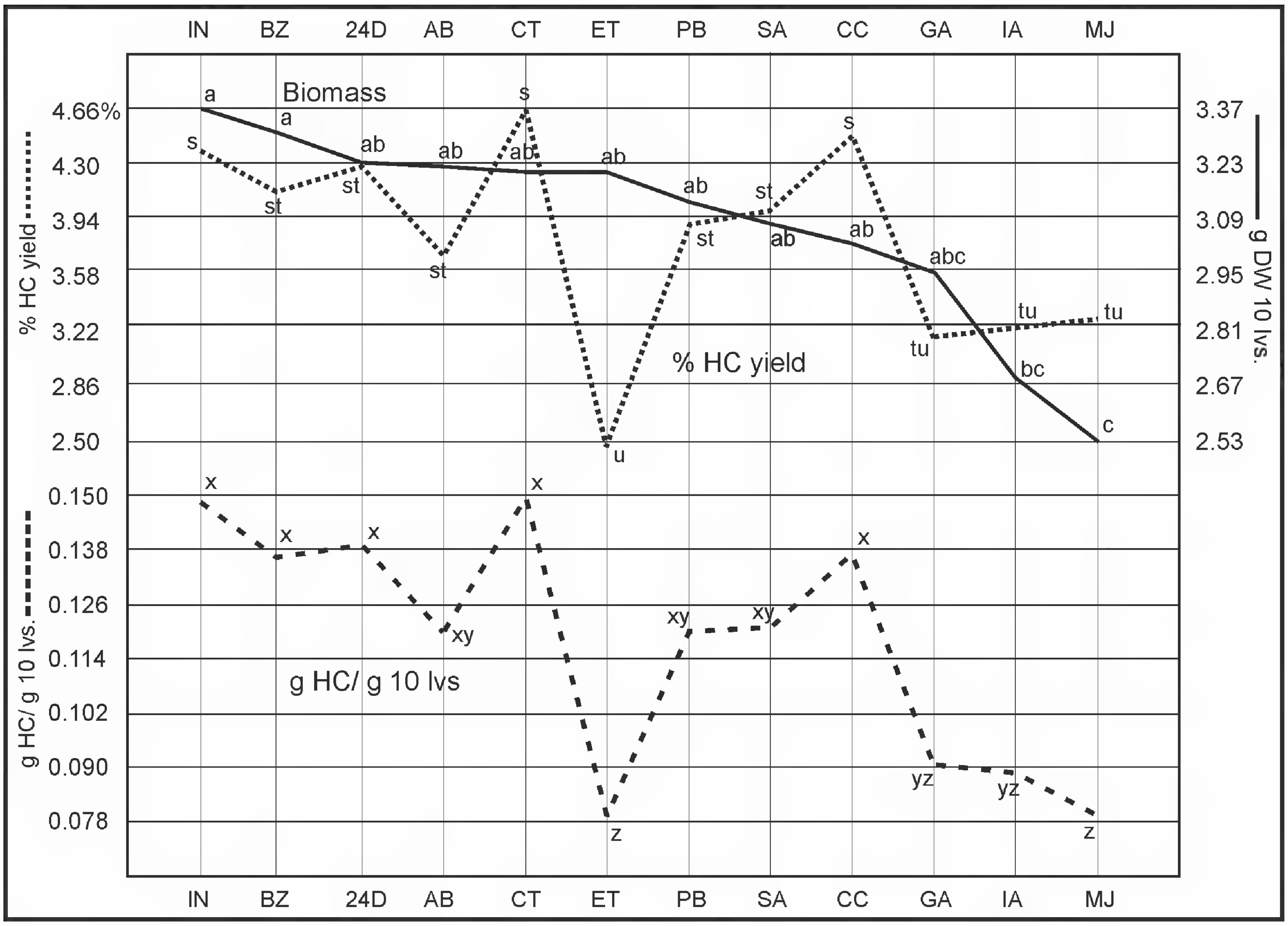

Figure 2. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Munchkin subjected to 12 treatments and analyzed 4 days later. Means with the same letter superscripts are not significantly different (P= 0.05). See text for discussion.

This research was initiated in the hope of stimulating the production of free HC for use as fuels from sunflowers. However, none of these treatments increased the production of HC in 4 days in cv. Munchkin. This may be due to the mixture of chemical classes such as terpenoids, lipids, waxes and sterols that are controlled by genes in various conflicting pathways. However, it should be noted that sunflowers growing in harsh, ambient conditions did produce very, much higher yields of HC than when grown in the greenhouse (Fig. 1). So, it still appears that some exogenous factor induces increase HC yields in sunflowers, although we did not discover the factor(s) in this study.

ACKNOWLEDGEMENTS

Thanks to Connie Stratton for care in growing the plants. This research supported by funds from Baylor University (0324512 to RPA).

LITERATURE CITED

Adams, R. P., M. F. Balandrin, K. J. Brown, G. A. Stone and S. M. Gruel. 1986. Extraction of liquid fuels and chemical from terrestrial higher plants. Part I. Yields from a survey of 614 western United States plant taxa. Biomass 9: 255-292.

Adams, R. P. and G. J. Seiler. 1984. Whole plant utilization of sunflowers. Biomass 4:69-80.

Adams, R. P. and A. K. TeBeest. 2016. The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*. Phytologia 98: 213-218.

Adams, R. P., A. K. TeBeest, B. Vaverka and C. Bensch. 2016. Ontogenetic variation in hexane extractable hydrocarbons from *Helianthus annuus.* Phytologia 98: 290-297

Adams, R. P. and A. K. TeBeest. 2017. The effects of different concentrations of gibberellic acid (GA3) on seed germination of *Helianthus annuus* and *H. petiolaris* Phytologia 99: 32-35.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet, C. Parker and D. Thornburg. 2017a. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 1-10.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet and D. Thornburg. 2017b. Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 130-138.

Adams, R. P., A. K. TeBeest, T. Meyeres and C. Bensch. 2017c. Genetic and environmental influences on the yields of hexane extractable hydrocarbons of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99(2): 186-190.

Adams, R. P., A. K. TeBeest, S. McNulty, W. H. Holmes, J. A. Bartel, M. Corbet, C. Parker, D. Thornburg and K. Cornish. 2018a. Geographic variation in natural rubber yields in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 100: 19-27.

Adams, R. P., Matt Lavin and Gerald P. Seiler. 2018b. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II. Phytologia 100(2): 153-160.

Adams, R. P., Matt Lavin, Steve Hart, Max Licher and Walter Holmes. 2018c. Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani* and *H. nuttallii* (Asteraceae). Phytologia 100(2): 161-166.

Browse, J. 2005. Jasmonate: an oxylipin signal with many roles in plants. Plant Hormones 72: 431-456.

Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoid accumulation in Cotton (*Gossypium hirsutum*) foliage is a general wound response. J. Chem. Ecol. 34: 508-522.

Majdi, M., M. R. Abdollahi and A. Maroufi. 2015. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in *Tanacetum parthenium*. Plant Cell. Rep. DOI 10.1007/s00299-015-1837-2.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath and M. Whalen. 2010a. Natural rubber quantification in sunflower using automated solvent extractor. Indust. Crops and Prods. 31: 469-475.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath, J. L. Brichta and J. E. van Fleet. 2010b. Agronomic and natural rubber characteristics of sunflower as a rubber-producing plant. Indust. Crops and Prods. 31: 481-491.

Rowe, H. C., Ro, D-K and L. H. Rieseberg. 2012. Response of Sunflower (Helianthus annuus L.) leaf surface defenses to exogenous methyl jasmonate. PLoS ONE 7(5): e37191. doi:10.1371/journal.pone.0037191.

Seiler, G. J., M. E. Carr and M. O. Bagby. 1991. Renewables resources from wild sunflowers (*Helianthus* spp., Asteraceae). Econ. Bot. 45: 4-15.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York.

Whittstock, U. and J. Gershenzon. 2002. Constitutive plant toxin and their role in defense against herbivores and pathogens. Curr. Opin. Plant Biol. 5: 300-307.